Drug Testing in the Neonate

Steven W. Cotten, PhD, DABCC

INTRODUCTION

A major portion of toxicology testing deals with urine drug screening for the adult population. This group can be divided into two major applications: preemployment urine drug screening and periodic scheduled pain management screening. Pain management clinics usually require patients to sign opiate contracts that allow for regular testing as a means to assess compliance. The presence or absence of drugs and metabolites must match the patient’s prescribed medications, and any discrepant compounds found during routine screening are grounds for dismissal from the pain management program. In addition to these two applications, toxicology drug testing plays an important but often overlooked role in newborn drug screening. Testing this population comes with its own set of unique analytical, therapeutic, and legal issues that can make screening and result interpretation challenging.

KEYWORDS

- Neonate  
- Drugs of abuse  
- Meconium  
- Pregnancy  
- Newborn

KEY POINTS

- Drug screening in the newborn population comes with a set of unique analytical, therapeutic, and legal issues that can make testing and result interpretation challenging.
- Assessment of in utero drug exposure to cocaine, amphetamines, opiates, marijuana, and ethanol may allow better intervention and management of withdrawal symptoms for the neonate.
- A range of maternal and neonatal specimens are available, but each comes with a unique set of limitations regarding sensitivity, invasiveness, and window of detection.
- Preanalytical issues such as specimen collection and sample extraction can influence test accuracy, and the particular biological specimen evaluated determines the window of detection achieved.
- Meconium provides the longest window, but the extraction technique greatly impacts sensitivity, and unique drug metabolites in meconium may lead to discrepancies between maternal and neonate results.

The author has nothing to disclose.
Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, 101 Manning Drive, Chapel Hill, NC, 27514, USA
E-mail address: scotten@unch.unc.edu

0272-2712/12/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
The rationale for newborn drug screening seeks to establish a picture of prenatal drug exposure during pregnancy. Conventional testing in adults uses both self-reporting and biological specimen testing to provide the necessary information for establishment of compliance or abuse. In the case of newborns, self-reporting is not applicable, and clinicians must rely on information from the mother coupled with biological testing from both the mother and neonate. Additionally, interpretation of drug screening results may be left to physicians, nurses, or social services workers. As the complexity of testing and interpretation increases, it is imperative that the laboratory be proactive in educating the necessary parties involved, particularly those without extensive toxicology training, to ensure proper medical and legal decisions are made with the information provided.

The unique analytical and legal caveats associated with newborn drug testing pose a variety of challenges to laboratorians and clinicians alike when it comes to screening this specific population. Preanalytical issues such as specimen collection and sample extraction can influence test accuracy. Samples can easily be adulterated because of the access of family members to the infant and extensive unsupervised time. Newborns frequently have dilute urine, so false-negatives are likely. Furthermore, sample volume is often low, limiting the ability for comprehensive screening and confirmation testing. Therefore, negative urine results do not definitively rule out drug exposure.

Positive urine results cannot distinguish between intermittent and chronic use by the mother. Often there is low agreement between specimens from the mother and newborn as well as results from screening and confirmation. Instances of discordant results complicate interpretation, particularly in cases with multiple specimens. Several therapeutic issues related to medical management are also unique to this population. The mother may initially delay prenatal care to avoid urine drug testing. Subsequent toxicology results have limited predictive value on genetic changes that have taken place earlier during the pregnancy. Positive drug screening results, however, can allow for proper medical management of withdrawal symptoms for certain drug classes.

Perhaps the most serious issues related to newborn drug testing are the legal implications surrounding decisions made in the case of positive results. Positive urine or meconium drug samples in newborns trigger involvement from social services for assessment of child safety. Twelve states formally consider positive urine drug screen in an infant to be child abuse; therefore, laboratory results can potentially remove newborns from their biological parents and place them in the foster care system. For this reason, the caveats and limitations of drug testing in this population are of utmost importance.

**SUBSTANCE ABUSE DURING PREGNANCY**

Accurate assessment of substance abuse during pregnancy is challenging for clinicians and other health care workers. Determination of abuse can come from either self-reporting or biological specimen testing. The US Department of Health and Human Services in conjunction with the Substance Abuse and Mental Health Administration periodically conducts surveys on drug use in persons 12 years and older in the general US population. In 2009, data estimated 22.6 million Americans (8.9%) used illicit drugs in the month before the survey. A total of 4.4% of pregnant women ages 15 to 44 admitted to substance abuse in the last month. This rate was lower than nonpregnant women (10.9%) and decreased with the age of respondent. The frequency of substance abuse was 16.2% for women ages 15 to 17, 7.4% for ages 18 to 25, and 1.9% for ages 25 to 55. Alcohol use among pregnant women was
estimated at 10.8%, with 3.7% admitting to binge drinking and 1.9% admitting to heavy alcohol use.

When biological specimens from pregnant mothers or newborns are tested for the presence of drugs, the rate of substance abuse detected increases. Using biological specimens, the frequency of illicit drug use in pregnant women has been estimated at 20% using maternal urine, maternal hair, newborn urine, or meconium.\textsuperscript{2–4} Tobacco and alcohol use/abuse are also estimated around 19%.

**CRITERIA FOR TESTING AND CONSENT**

Clinical indication for substance abuse testing is highly variable between regions. Currently there are no federal guidelines defining criteria for the testing of newborns or pregnant women. Therefore, it is up to individual institutions and health care systems to draft explicit guidelines for newborn and maternal drug testing to best identify and manage substance abuse in their specific population. Evidence-based clinical practice guidelines have been developed to provide recommendations for standardized screening approaches.\textsuperscript{3,5,6} Typical institutional guidelines for toxicology screening for newborns may resemble the following. (Adapted from the newborn nursery guidelines from the University of North Carolina at Chapel Hill.\textsuperscript{7})

Urine or meconium drug testing is clinically indicated by the following:

- **Maternal History**
  - History of drug abuse.
  - Prenatal care starting after 16 weeks or less than a total of four prenatal visits.
  - History of child abuse, neglect, or court-ordered placement of children outside the home.
  - History of domestic violence.
  - History of hepatitis, human immunodeficiency virus, syphilis, or prostitution.
  - Unexplained placental abruption.

- **Infant History**
  - Unexplained intrauterine growth restriction.
  - Infants with evidence of drug withdrawal (hypertonia, irritability, or tremulousness).

- **Alcohol**
  - Acute maternal alcohol intoxication is observed around the time of delivery.

Consent for collection is an additional issue requiring special attention for toxicology testing in this population. Informed consent may be required for both maternal and newborn specimens depending on institutional and state guidelines. Consent for collection of newborn urine or meconium for drug screening is often covered under the general consent for treatment for most health care institutions. Departments of obstetrics and gynecology within the hospital may seek separate verbal consent from the mother and inform the parents prior to testing if drug screening becomes clinically indicated.

**LEGAL IMPLICATIONS**

Most clinical laboratories do not routinely perform chain of custody for specimens submitted for toxicology analysis. This protocol differs from employment urine drug screening where documentation of chain of custody is necessary for validity of the results. Legal action can be taken only if chain of custody is properly documented. In the case of newborn drug screening, if a specimen is positive for an illicit substance, chain of custody is not required for legal action or involvement by social services. This difference illustrates an important nuance in newborn drug testing in that both
medical and legal decisions are made using the results generated by the clinical laboratory. It is therefore imperative that all parties involved in the testing process—nurses, medical technologists, physicians, laboratory directors, substance abuse counselors, and social services workers—have a unified protocol for testing and understand the limitations.

A compendium of individual state laws regarding parental drug use and child abuse is available from the Child Welfare Information Gateway. Arkansas, Colorado, Florida, Illinois, Indiana, Minnesota, North Dakota, South Carolina, South Dakota, Texas, Virginia, Wisconsin, and the District of Columbia all consider a positive newborn urine drug screen in their definition of child abuse or neglect (Fig. 1). An additional 13 states have specific reporting procedures for newborns that show evidence of exposure to drugs or alcohol. The Child Abuse Prevention and Treatment Act requires states to develop formal policies for informing child protective services in cases where newborn drug exposure is documented and to develop plans for protective care and medical management of withdrawal symptoms.

**TYPES OF SPECIMENS**

The goal of toxicology drug testing in newborns is to evaluate in utero drug exposure during the course of the pregnancy. A wide range of maternal and neonate biological specimens are available to clinicians. Each specimen comes with its own unique set of limitations regarding sensitivity, invasiveness, and window of detection. Biological matrices from the newborn include meconium, hair, cord blood, and neonate urine. Specimens from the mother include hair, blood, oral fluid, sweat, urine, and breast milk. Matrices that contain drugs and metabolites from both the mother and neonate include the placenta and amniotic fluid. In order to develop a complete understanding of total drug exposure during pregnancy, clinicians must consider the type of specimen being tested, the type of drug expected, and the window of detection (Fig. 2). The rate of agreement between mother and infant can often be low because of these complicating factors.

**SPECIMENS FROM THE NEONATE**

The three types of commonly used specimens from the newborn include neonate urine, neonate hair, and meconium. Neonate urine is the most frequently used
specimen to assess in utero drug exposure. Despite its popularity, urine samples provide the shortest window of detection, only capturing drug use several days before delivery. Newborns also tend to have dilute urine, so false-negatives are likely.

Neonate hair forms during the last trimester, and therefore can capture drug exposure for the last 3 to 4 months during pregnancy. The parent compounds of drugs typically accumulate in hair rather than the more polar metabolites found in blood, urine, and meconium through either passive diffusion from arterial blood capillaries of the hair follicle or excretion on the surface of the head. Typically 20 to 50 mg of hair are needed for adequate testing, but because quantities may be limited in newborns and the procedure is considered partially invasive, adoption of hair testing has not gained routine usage.

Meconium refers to the first fecal matter passed during the first days of life; it is characterized by a dark green/black color. Formation of meconium begins around the 12th week of gestation and continues to accumulate until birth. Drugs of abuse are deposited in meconium through absorbance across the placenta, metabolism by the fetal liver, and swallowing of amniotic fluid. The length of accumulation imparts the largest window of detection for drugs of abuse for any neonate specimen, theoretically capturing the entire profile of exposure over the last two trimesters.

Collection of meconium is considered noninvasive, with 99% of full-term infants passing their entire meconium after 48 hours. Drug-exposed neonates show a slower evacuation of meconium, with the median first stool passing at day 3 and 90% by day 12. For analysis, a minimum of 0.5 grams is collected and stored at $-20^\circ C$ to $-80^\circ C$ prior to organic solvent extraction and measurement. The choice of solvent depends on the compound measured and the subsequent method used for analysis. Methanol is the most common extraction solvent for cocaine, opiates, cannabinoids, and amphetamines when liquid chromatography–mass spectrometry (LCMS) is used, with extraction efficiencies between 40% and 80% depending on the individual metabolite assayed. Extraction under acidic conditions may increase recovery through hydrolysis of glucuronide metabolites at a low pH. The extraction procedure for recovery of drug metabolites from meconium samples has a much greater effect on assay sensitivity than the screening or confirmation procedure itself.

Fig. 2. Window of detection for biological specimens: The window of detection varies depending on the sample chosen for drugs of abuse screening. (From Lozano J, García-Algar O, Vall O, et al. Biological matrices for the evaluation of in utero exposure to drugs of abuse. Ther Drug Monit 2007;29:711–34, Figure 2; with permission.)
SPECIMENS FROM THE MOTHER

Biological specimens available for toxicology testing from the mother include maternal hair, urine, blood, amniotic fluid, placenta, cord blood, and oral fluid. Of these specimens, maternal hair and urine are used most frequently during pregnancy and immediately after delivery to assess drug use by the mother. Maternal blood only captures acute consumption occurring a few days or hours prior to collection and has found limited value in testing for drugs of abuse. Oral fluid is noninvasive compared with blood, and drugs that are weak bases such as cocaine, opiates, benzodiazepines, and nicotine tend to accumulate in oral fluid relative to serum. The distribution, detection window, and analyte stability in oral fluid are still unknown for many drugs of abuse.\(^\text{17,18}\) Generally only parent compounds are the major component found in oral fluid, and the specimen only detects acute consumption hours before collection.

The placenta is the maternal organ that forms after the fourth week of pregnancy and acts as the interface between maternal and fetal blood, allowing for exchange of nutrients and waste during development. Metabolism and degradation of xenobiotics by the placenta can also occur and impact drug transfer to fetus. Biomarkers of ethanol use by the mother, namely fatty acid ethyl esters (FAEEs), are not transferred to the fetus but are instead absorbed and degraded by the placenta.\(^\text{19}\) Therefore, any FAEEs detected in the neonate are a result of direct exposure to ethanol and metabolism by the fetus in utero. Despite its important role in drug metabolism and exposure during pregnancy, placenta testing remains an esoteric specimen used in toxicology testing.

Amniotic fluid accumulates throughout pregnancy, having a volume of approximately 30 mL at week 10 and up to 1000 mL by week 37.\(^\text{20}\) The highly aqueous nature of the specimen selectively enriches for water-soluble drugs. Collection of amniotic fluid, however, can be dangerous for the fetus, and its use for drug testing is not routine. Despite this obstacle, several studies have used amniotic fluid to confirm cocaine exposure, but its popularity as a biological fluid for prenatal drug use remains low.\(^\text{21–24}\)

The two most popular biological specimens from the mother for assessing prenatal drug exposure are maternal hair and urine. Maternal hair captures chronic drug use over the longest period of time. Collection is noninvasive and provides a direct estimate of maternal drug exposure throughout the entire pregnancy or before. Drug deposition in the hair may, however, be affected by hair care products or cosmetic hair treatments that damage the follicle or hair proteins.\(^\text{25}\) Maternal hair analysis provides only an indirect estimation of drug exposure for the fetus. Several studies have attempted to correlate cocaine concentrations between maternal and neonate hair with unpredictable results.\(^\text{26}\) Maternal urine in conjunction with neonate urine is probably the most popular specimen to assess drug exposure several days before delivery. Despite its short window of detection, collection is easy, extraction is efficient, and sensitivities in the ng/mL can be achieved for most drugs tested using LC or gas chromatography–mass spectrometry (GCMS).

AGREEMENT BETWEEN MATERNAL AND NEONATE SPECIMENS

Immunoassay screening and mass spectrometry (MS) confirmation results for both maternal and neonate specimens, along with maternal self-reporting, may show varying degrees of agreement. Several factors contribute to these discrepancies. Maternal self-reporting is estimated to capture only 4% of illicit drug users from a general population. Urine specimens from both the mother and neonate have a narrow window of detection for most drugs and are greatly affected by hydration.
status. In the case of meconium, extraction efficiency and completeness of collection may negatively select for drug detection. Furthermore, if GCMS is used for confirmation, extraction, derivatization, and volatilization will not be equal for different drug classes and their respectively glucuronidated metabolites. Comparing results from different matrices (maternal urine vs neonate meconium) often shows the presence of certain drugs in one specimen that are absent in another.

SPECIMEN COLLECTION AS A SOURCE OF PREANALYTICAL VARIABILITY

In addition to distinctive specimens used in newborn testing, collection methods for urine and meconium are unique. These newborn-specific factors may contribute to preanalytical variability that can affect assay results. The physical collection of urine specimens from newborns is often overlooked as a source of preanalytical variability. A recent study illustrated a wide range of collection procedures in place for neonate urine collection at one institution. Nursing staff at this institution used specially designed urine bags, diapers turned inside-out, cotton balls, or gauze followed by syringe extraction from the textile matrix to collect the urine specimen prior to sending the sample to the laboratory. In some instances the infant was given a bath prior to application of the collection device, whereas other times baby wipes were used. As a result a variety of textiles including diapers, baby wipes, gauze, and lotions could potentially come in contact with the sample prior to analysis.

It is therefore important to validate the collection procedure to identify interferences that may generate false-positives or false-negatives. A false-positive interference from baby wash soaps has recently been reported for several tetrahydrocannabinol (THC) immunoassays for cannabinoids. The interferent was identified after an increase in positive specimens was observed specifically from the institution’s newborn nursery. This result can be problematic because confirmatory testing is not mandated in the clinical setting. The interferent was found to be analyte- and manufacturer-dependent. A variety of other household chemicals, namely liquid soap and Visine, have previously been reported as positive interferents in drugs of abuse assays in the context of adulterants for preemployment screening but not in the newborn population.

METHODS FOR SCREENING AND CONFIRMATION

Conventional urine drug toxicology consists first of a broad screening method that detects a class of related compounds followed by confirmation of positive results with a second method that detects a defined list of compounds within that class. Regardless of the matrix, urine drug screening almost exclusively uses immunoassay-based platforms that target cocaine, amphetamines, cannabinoids, opiates, or phencyclidine. Clinical laboratories confirm presumptive positive results using MS coupled with LC or GC that imparts increased sensitivity and specificity for quantitating a specific set of compounds. Given the medical and legal decisions that potentially impact patient care and the unique specimens associated with neonate drug screening, it is recommended that all toxicology screening results for drugs of abuse be confirmed.

SPECIFIC DRUGS OF ABUSE

Cocaine

Illicit drug use including cocaine among pregnant women is estimated at 4% based on national studies. After ingestion via smoking or insufflation, cocaine enters the blood where it can be metabolized by the liver or rapidly cross the placenta via
Cocaine Metabolism

Fig. 3. Cocaine metabolism: The cocaine metabolites present in a sample are dependent on concomitant ethanol use, route of administration, and the biological specimen evaluated.

passive diffusion. Several important metabolic pathways for cocaine are recognized (Fig. 3). The various metabolites have a diverse range of polarities and therefore are not uniformly enriched in meconium.30,31 Cocaine, norcocaine, p-hydroxycocaaine, benzoylecgonine, ecgonine methyl ester, ecgonine, anhydroecgonine methyl ester, cocaethylene, and norcocaethylene are the major metabolites frequently found in meconium. Interestingly, ecgonine has been shown to occur at the highest frequency and median level for cocaine-exposure infants using solid phase extraction of the meconium.30 Evaluation of cocaine metabolism for either meta or para hydroxylation showed para hydroxylation to occur at a higher frequency in samples. Median values for meta hydroxylation (m-hydroxycocaaine or m-hydroxybenzoylecgonine), however, exceeded median values for their corresponding para metabolites when present.30

Concomitant use of ethanol and cocaine generates the ethyl derivatives norcocaethylene, cocaethylene, and ecgonine ethyl ester via transesterification with ethyl alcohol instead of water. Two specific metabolites can be used to differentiate
insufflated cocaine from smoked (crack) cocaine. The pyrolytic products anhydroecgonine methyl ester and anhydroecgonine are both generated directly from the loss of benzoic acid by cocaine during volatilization. The frequency of detection for both ethanol- and crack cocaine–associated metabolites is greater than 95% in the meconium of infants whose mother’s urinalysis was positive for benzoylecgonine.30

Conventional methods for both immunoassay and MS confirmation target benzoylecgonine, the major ionic metabolite of cocaine found in urine. Current immunoassays effectively recognize both benzoylecgonine and \( m \)-hydroxybenzoylecgonine but show little reactivity toward cocaine, ecgonine methyl ester, ecgonine, or cocaethylene.32 This result may account for some discrepant values between neonate and mother dyads in regard to positive or negative result agreement.

Methanol extraction of meconium is frequently used for isolation of cocaine metabolites. This extraction can be coupled with solid phase extraction to further enrich additional compounds other than benzoylecgonine through reconstitution in acidic buffer followed by additional washing and elution in methylene chloride/2-propanol-ammonium hydroxide (78/20/2).30 This inclusive extraction procedure has reported recoveries of 15 unique cocaine metabolites between 38.9% and 59.1%.

Immunoassay-based screening methods including radioimmunoassay, enzyme-multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay, and fluorescence polarization have all been used for cocaine determination with neonate urine and meconium.12 GC and LCMS methodologies have been adopted for confirmation testing of meconium samples allowing for quantitation of comprehensive panels of cocaine metabolites. LCMS methods achieve greater sensitivity than GCMS because of elimination of the derivatization and volatilization steps required by gas chromatography.

Effects of in utero cocaine exposure
A large epidemiologic study of 11,811 mother-infant pairs compared meconium and maternal self-reporting to assess drug exposure.33 Of the analyzed samples, 9.5% were positive for cocaine or its metabolites using EMIT immunoassay followed by GCMS. At birth, neonate exposure to cocaine was significantly associated with low birth weight and altered length and head circumference from normal ranges.34 Additionally, prenatal cocaine exposure has been linked to delayed language development, decreased total language performance, behavior problems in school, and increased risk of obesity through early adolescence.35,36

Cannabinoids
Cannabinoids are a class of compounds produced by Cannabis sativa and Cannabis indica with \( \Delta^9 \)-THC being the most prevalent and the major psychoactive component. Assessment of prenatal cannabis exposure relies on maternal self-reporting, maternal urine, neonate urine, and meconium. Metabolism of THC generates 11-nor-\( \Delta^9 \)-tetrahydrocannabinol-9-carboxylic acid (THC-COOH) through the active intermediate 11-hydroxy-\( \Delta^9 \)-tetrahydrocannabinol (11-OH-THC). The majority of immunoassays target THC-COOH because it is the major constituent in cannabinoid-positive urine samples.37 Meconium does, however, have a greater likelihood of detecting sporadic cannabis use during pregnancy; it is often preferred over newborn urine (Fig. 4).

Differences in cannabinoid metabolite content exist between urine and meconium in the neonate that can lead to high rates of false-negatives. Studies evaluating meconium samples that screen positive by EMIT assay but confirm negative for THC-COOH revealed a 40% false-positive rate.38 Further analysis of meconium
samples that screened negative for THC-COOH detected 11-OH-THC and 8β-11-dihydroxy-Δ9-tetrahydrocannabinol (8β-di-OH-THC) instead. These data suggest that meconium may selectively enrich distinct cannabinoid metabolites from urine, and that confirmation rates can be increased by incorporation of these additional compounds (see Fig. 4).

A dose-response relationship between maternal drug use and neonate meconium cannabinoid concentration has not been established, making estimation of maternal episodic use difficult. Serial collection of meconium samples over the first several days has demonstrated a 60% likelihood of a subsequent positive result if the first collected sample is positive.14 This issue highlights the heterogeneous nature of meconium collection and how the dynamics of gastrointestinal motility can affect test results.

Sample preparation for cannabinoid extraction from meconium can be achieved using normal saline, methanol, acetic acid/diphenylamine, or hexane/ethyl acetate. Methanol extraction and subsequent analysis of THC-COOH can yield recoveries between 50% and 72%. THC and its metabolites are glucuronidated in both urine and meconium; therefore, enzymatic, acid or base hydrolysis improves sensitivities for GC or LC-MS.

Interferences in THC-COOH immunoassay assays have been reported on a variety of manufacturer platforms. Newborn-specific factors related to sample collection (urine) may also impart preanalytical variation for THC immunoassay results. To complicate immunoassay interpretation there are three prescription drugs that contain cannabinoid-based molecules. Medicinal marijuana is available by prescription in one out of three states in the United States, the pain management drug Marinol (THC) is given to specific pain populations, and the new neurologic agent (Sativex, THC/cannabidiol) is used for the treatment of multiple sclerosis. Although there are no
recommendations for use of the medications during pregnancy, drug screening results should be critically evaluated if the mother has a medical history involving any of these prescription drugs.

**Effects of in utero cannabinoid exposure**

Numerous studies have sought to measure quantitatively the effects of maternal cannabis use on neonate development. Several studies have reported negative physical effects on birth weight, length, and gestational age.\(^{45,46}\) Below-normal performance on intelligence tests, increased frequency of depression, and greater likelihood to use cannabis in adolescence have also been associated with prenatal exposure.\(^{47-49}\) Yet other studies find no adverse effects at birth or demonstrable long-term differences between exposed and nonexposed neonates.\(^{50-52}\) Such discrepant findings have been suggested to arise from inability to properly stratify exposed from nonexposed infants.\(^{53}\) Despite a lack of conclusive evidence for or against harmful effects of prenatal THC exposure, substance abuse often correlates with other environmental and social factors that can negatively affect development.

**Opiates**

The frequency and amount of opiate consumption has increased dramatically in the past decade. Drug screening has become increasingly complex as the need to discriminate between over-the-counter, prescription, and illicit opiates grows in the clinical setting. The rapid emergence of pain management clinics now requires specific quantitation of multiple prescription opiates to assess patient compliance. In the setting of pregnancy, opiate testing is important for both maternal and neonate health, particularly for opiate-dependent mothers and the proper management of neonate abstinence syndrome at birth.

The type of opiate molecule in question will determine its immunoassay reactivity, transport across the placenta, and tissue distribution in the neonate. Morphine-based opiates such as morphine, codeine, oxycodone, hydrocodone, buprenorphine, and heroin are lipophilic and will be transferred readily across the placenta to the fetus. Their corresponding glucuronidated metabolites are more hydrophilic and will cross the placenta via diffusion-limited transfer.\(^{54}\) The rate of diffusion is gated by the size of the molecule as well as the permeability of the placenta, which changes with gestational age. Based on animal studies, synthetic opiates such as fentanyl, methadone, tramadol, and loperamide may exhibit greater transfer across the placenta than morphine-based drugs and subsequent increased fetal exposure.\(^{55}\)

Opiate metabolism generates glucuronidated compounds that are found in both urine and meconium. Neonate urine samples provide a narrow window of detection of maternal drug use in the past 3 to 7 days. For heroin, the generation of 6-monooacetylmorphine is only detectable hours after use before the intermediate is converted entirely to morphine. The major cyclic metabolite of methadone, 2-ethylidene-1,5-dimethyl-3,3-diphenyl-pyrrrolidine (EDDP), has been detected in meconium using fluorescence polarization and high-performance LC with diode array detection.\(^{56}\) A correlation was seen with the maternal methadone dose and the amount of meconium methadone measured but not with the methadone metabolite EDDP.

Immuoassay reactivity toward opiates varies depending on manufacturer, platform, and target compound. Semisynthetic morphine-based opiates such as oxycodone and buprenorphine may show partial reactivity, whereas nonmorphine-based opiates such as methadone and fentanyl may not react at all. Methanolic extraction of meconium is frequently used for isolation of opiates from the specimen prior to
analysis. Extensive coverage of the methodologies and detection limits for a variety of opiate assays in the setting of neonate drug testing has been reviewed elsewhere.\textsuperscript{12}

**Neonate abstinence syndrome**
Methadone is currently the only approved medication for use during pregnancy by opioid-dependent women.\textsuperscript{57} Maintenance therapy for pregnant women is thought to reduce illicit drug-seeking behavior, minimize withdrawal-induced stress on the fetus, and improve prenatal care. Maternal methadone use exposes the neonate to opiates during gestation, which can present central and autonomic nervous system dysfunction at birth, frequently called neonate abstinence syndrome (NAS). Assessment depends on observed behaviors such as excessive crying, poor sleep, tremors, increased muscle tone, generalized seizure, hyperthermia, tachypnea, poor feeding, failure to thrive, and irritability. Pharmacologic management depends on degree of severity, with 52\% of NAS patients requiring only opiates for treatment.\textsuperscript{58} Second line therapies include phenobarbital and opiates in 32\% of cases. Buprenorphine has recently been reported as a safe and effective treatment for NAS, along with clonidine.\textsuperscript{59,60}

**Amphetamines**
Amphetamine drug testing in the neonate is similar to opiate testing in that it must discriminate between over-the-counter, prescription, and illicit compounds within the same class. Immunoassay screening serves only to capture the maximum number of true-positives for reflex confirmation testing while minimizing false-positives. Specificity of reagents and platforms varies for amphetamine drugs of abuse detection. It is therefore up to laboratories to educate health care professionals in proper test utility and institution-specific limitations.

Methamphetamine abuse has increased sharply in specific regions of the country over the last 10 years, with 11,239 clandestine laboratories discovered in 2010.\textsuperscript{61} Tennessee, Kentucky, and Illinois account for 30\% of the total manufacturing of methamphetamine in the United States. Approximately 17\% to 44\% of laboratories have children living in the same building. In the last several years, a shift in the synthetic route used for the manufacturing of methamphetamine has occurred that allows for small-scale mobile manufacture in 2 L bottles. The method known as “shake and bake” has increased the number of amateur producers and subsequently burn victims admitted to hospitals.

Maternal use of methamphetamine increased from 8\% in 1994 to 24\% in 2006 for pregnant women positive for an illicit substance.\textsuperscript{62} Methamphetamine crosses the placenta within 30 seconds of injection in animal studies and exhibits slower elimination in the fetus resulting in longer exposure. Heavy prenatal use of methamphetamine is associated with high concentrations (200–1000 ng/g) in meconium. Amphetamines, including methamphetamine, MDMA (methylenedioxymethamphetamine), and MDA (methylene dioxyamphetamine) can be deaminated or hydroxylated in the liver to inactive metabolites. Unlike other drug classes, a significant portion (30\%–40\%) is excreted unchanged in the urine, making detection of the parent compound acceptable for assessing exposure. Detection in neonate urine captures exposure several days before birth. False-positive results from labetalol, a drug used to treat hypertension during pregnancy, have been reported, highlighting the need for confirmation of positive immunoassay screening results from maternal urine.\textsuperscript{63}

Methanolic extraction often coupled with solid phase purification is used for extraction of amphetamines from meconium.\textsuperscript{42} Confirmation by GC and LCMS offers sensitivities in the ng range per gram of meconium. Extraction efficiencies between
40% and 87% have been reported across a wide range of concentrations. Additionally, specific byproducts of methamphetamine production have been used in the forensic setting to determine which route was used for synthesis.61,64

Side effects of prenatal methamphetamine and amphetamine exposure include decreased gestational age, birth weight, length, and occipitofrontal circumference.62 Additionally, infants are more likely to be admitted to neonate intensive care units after birth. Beyond initial clinical effects, decreases in mother-infant bonding, decreases in breast-feeding, higher adoption rates, neglect, and court-ordered placement outside the home are associated with mothers who use methamphetamine.65

Ethanol

Assessment of ethanol abuse during pregnancy is challenging because of rapid metabolism and elimination by the body.66 The pseudo-zero order kinetics and rapid distribution throughout tissues make detection in the blood and urine difficult. Diagnostic tests to assay neonate exposure to alcohol are currently evaluating the emerging biomarkers of FAEEs, which are produced through nonoxidative breakdown of ethanol (Fig. 5).67 Normal reaction of fatty acids with glycerol in the body produces monoglycerides, diglycerides, and triglycerides that act as secondary messengers and cellular components. In the presence of ethanol, fatty acids form FAEEs through a condensation reaction. Once formed, FAEEs are deposited in fatty tissues and meconium, thereby serving as markers for long-term alcohol exposure during pregnancy.
Studies evaluating transfer of FAEEs across the placenta demonstrate that FAEEs generated by maternal metabolism are broken down and degraded in the placenta. Accumulation of FAEEs in meconium, therefore, represents ethanol that has been transferred across the placenta and metabolized by the fetus in utero. The major FAEEs currently under evaluation include ethyl laurate, ethyl myristate, ethyl palmitate, ethyl palmitoleate, ethyl stearate, ethyl oleate, ethyl linoleate, ethyl alpha-linoleate, ethyl arachidonate, and ethyl heptadecanoate.

Correlation between maternal alcohol consumption and FAEEs levels in meconium show a positive relationship, but the specific analytes and cutoff levels defining exposure remain unclear. Total FAEEs levels are suggested to provide better predictive power for maternal alcohol abuse rather than select individual metabolites. Furthermore, the cutoff levels for positive prenatal alcohol exposure vary by study, with reported cutoffs ranging from 50 to 600 ng/g.

Extraction of FAEEs from meconium has been achieved with hexane/acetone or solid phase extraction with recoveries ranging from 20% to 93% depending on the specific metabolite tested. Testing methods have used GC–flame ion detector, full scan GC-MS, selected ion monitoring GC-MS, and GC-MS/MS for screening and confirmation of various analytes.

**Fetal alcohol spectrum disorder**
Prenatal exposure to alcohol can result in a broad array of detrimental effects to the developing fetus. Fetal alcohol spectrum disorder (FASD) is the umbrella term used to classify neonates that exhibit one or more of the features associated with ethanol exposure including growth retardation, abnormal facial features, and central nervous system impairment. Height and weight below the 10th percentile, short palpebral fissures, thin vermilion border, smooth philtrum, and impaired cognitive function are all used as criteria for diagnosis of FASD. It is estimated that the occurrence of FASD is between 0.2 and 2 cases per 1000 births in the United States. Secondary effects that may present later in life include legal trouble, mental health issues, behavioral problems, and low social adaptability. The cost of FASD has been estimated at $3.6 billion annually, illustrating its severity as a major public health issue.

**MANAGEMENT OF WITHDRAWAL FROM DRUGS OF ABUSE**

Guidelines for medical management for chronic prenatal exposure to opiates, benzodiazepine, and alcohol have been proposed by the SOGC (Table 1). Neonates showing abstinence syndrome from opiates can benefit from symptomatic therapy, including Gravol for nausea and vomiting and acetaminophen/nonsteroidal anti-inflammatory drugs for myalgias. Methadone and buprenorphine can be initiated to step-down opiate exposure over time. If methadone is unavailable, morphine 5 to 10 mg by mouth every 4 to 6 hours as needed can be substituted. For chronic benzodiazepine exposure, two-thirds to three-fourths of the equivalent adult dose (mg/kg) can be administered, tapering 10% of the dose per day. Chronic alcohol exposure withdrawal can benefit from thiamine, folic acid, diazepam, or lorazepam depending on severity, with close monitoring of electrolytes.

**FUTURE PERSPECTIVES**

The neonate population holds unique challenges in relation to assessment of drug exposure. The specimens available show distinct differences from adult samples regarding windows of detection and analyte deposition. Accurate results are imperative given the legal and medical management issues related to newborn care.
Current research in the area of FAEEs may provide better tools to evaluate prenatal alcohol exposure in the future. Several additional facets of neonate drug testing deserve more research to understand exposure rates more clearly and detrimental effects of drugs during development. To date, little information exists regarding the frequency and level of the cocaine adulterant levamisole in meconium. Current estimates for levamisole prevalence in the adult population are approaching 100% for patients who screen positive for benzoylecgonine. Additionally, the synthetic cannabinoïds and novel ketone amphetamines currently emerging in adult populations have not been evaluated in the neonate population.

**REFERENCES**


---

### Table 1

**Management of withdrawal symptoms**

<table>
<thead>
<tr>
<th>Substance</th>
<th>Recommended Management</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Thiamine 100 mg po od × 3 d, folic acid 5 mg po od. Diazepam 20 mg po q 1–2 h until minimal symptoms. Lorazepam 2–4 mg sl/po q 2–4 h prn during labor. Monitor hydration status.</td>
</tr>
<tr>
<td>Benzodiazepines</td>
<td>Start at two-thirds to three-fourths of diazepam equivalent dose. Taper by 10% per day.</td>
</tr>
<tr>
<td>Opiates</td>
<td>Offer symptomatic therapy including Gravol for nausea and vomiting, acetaminophen/NSAIDs for myalgias. Consider methadone or buprenorphine initiation. Can use morphine 5–10 mg po q 4–6 h prn if methadone is not available.</td>
</tr>
</tbody>
</table>

**Abbreviations:** NSAIDs, nonsteroidal antiinflammatory drugs; od, once daily; po, by mouth; prn, as needed; q, every; sl, sublingual.


Emerging Biomarkers of Intrauterine Neonatal and Pediatric Exposures to Xenobiotics

Kaitlyn Delano, BSc\textsuperscript{a,b}, Gideon Koren, MD, FRCPC, FACMT\textsuperscript{a,b,*}

INTRODUCTION

There are multiple definitions available for a biomarker, specific to how the biomarker is used. The official National Institutes of Health definition of a biomarker is “a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.” In the context of detecting external toxins after exposure during intrauterine life, biomarkers are critical, because many chemicals may not exist any more in the blood or urine of the neonate. Consequently, without the availability of appropriate

\textsuperscript{a} Department of Pharmacology, University of Toronto, 1 King’s College Circle, Toronto, Ontario M5S 1A8, Canada; \textsuperscript{b} Division of Clinical Pharmacology and Toxicology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario M5G 1X8, Canada
* Corresponding author.
E-mail address: gkoren@sickkids.ca

http://dx.doi.org/10.1016/j.pcl.2012.07.005 pediatric.theclinics.com

0031-3955/12/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
biomarkers, even potential toxic intrauterine exposures may be missed. Therefore, biomarkers must be able to generate relevant preclinical or clinical interpretations.\textsuperscript{2} The sensitivity and specificity of a biomarker are important, because biomarkers too sensitive or nonspecific may not detect exposures or effects that are clinically relevant.\textsuperscript{3}

In this review, we focus on biomarkers of internal dose, a subtype of biomarkers of exposure, which indicate the occurrence and extent of exposure to a compound or its metabolite(s).\textsuperscript{3} Measuring the amount of the compound or metabolite in a matrix allows for a measurement of the exposure, rather than only estimating it.\textsuperscript{3} By using biomarkers of both the compound and metabolite(s), more information concerning the exposure can be gathered and more accurate interpretations can be made by the clinician. When available, using multiple biomarkers in conjunction may provide more clinically relevant information about the exposure.

Illicit substance use in pregnancy is associated with significant maternal and neonatal morbidity and economic burdens to the health care system.\textsuperscript{4} Despite a potential increase in substance abuse during pregnancy, it remains underdiagnosed or completely undiagnosed, putting both the fetus and the mother at risk for long-term sequelae.\textsuperscript{5} Maternal self-report has been commonly used in the past to assess potential fetal exposure, but has been found to be unreliable and not correlate with exposure.\textsuperscript{6} Using biomarkers to detect use of drugs, alcohol, or environmental toxins can help determine the optimal management of the child.

**HAIR AND MECONIUM AS MATRICES FOR IN UTERO BIOMARKERS**

Most often, blood and urine are used to test for drug and alcohol use or exposure. Although these 2 matrices are well established, they provide information on only very recent use or exposure, because of the short elimination half-lives of most drugs of abuse.\textsuperscript{7,8} Longer-term, chronic exposure is not detected using blood or urine, requiring alternative matrices to capture this type of exposure. Hair and meconium, in neonates, have emerged as novel matrices that provide a wider window of detection. These 2 matrices can be tested to assess prenatal exposure to chemicals, including those resulting from maternal usage.

**Hair**

Hair follicle development occurs because of ectodermal and mesodermal interactions during epidermal development, beginning at approximately the eighth week of development.\textsuperscript{9} The base of the follicle, the dermal papilla, is derived from the mesodermal mesenchyme of the dermis, whereas the remainder of the hair follicle is derived from the ectoderm.\textsuperscript{9} The pigmentation of hair and skin is caused by melanocytes, which develop from the neural crest cells.\textsuperscript{9} Melanin pigments, eumelanin and pheomelanin, are synthesized and stored in the melanosomes. Eumelanin produces brown and black hair, whereas pheomelanin is responsible for red and blond hair. The proportion of these 2 melanin pigments is what dictates the final color of human hair.\textsuperscript{10} The appearance of hair follicles occurs at around the 10th week of fetal development, and continued differentiation results in the formation of various components of the follicle.\textsuperscript{9}

Three types of glands are associated with the hair follicle: sebaceous, apocrine, and sweat glands. The sebaceous gland, responsible for the production of sebum, develops on the side of the follicle and is associated with capillary networks, similar to the hair follicle. Sebum is composed of free and combined fatty acids and unsaponifiable material (eg, cholesterol and waxes).\textsuperscript{9,11} Apocrine glands secrete an oily, colorless substance directly into the follicle, and are localized in the axilla, eyelids,
and external auditory meatus. Sweat glands, located on most of the body surface, produce sweat, comprising mainly water and salts.

Hair growth occurs in a 3-phase cycle, consisting of anagen, catagen, and telogen phases. The anagen phase represents hair production and begins at the 15th week of fetal development, and the scalp of the fetus is completely covered with anagen phase follicles between the 18th and 20th week of gestation. This phase is characterized by a rapid proliferation of matrix cells, which fill the follicle bulb, extending through epithelial cells. The matrix cells are then keratinized, forming the strand of hair. Growth of the hair continues until expression of epidermal growth factors, resulting in apoptosis of follicular keratinocytes and melanocytes, or the catagen phase. During the catagen phase, the bottom of the hair fiber is fully keratinized. At this point, the hair follicle is dormant and in the telogen phase. The first full cycle of hair growth is complete between the 24th and 28th week of development, with the next cycle starting soon after the first one is completed. Consequently, biomarkers present in hair at birth reflect exposure to toxins during the third trimester, and are able to be tested until approximately 3 months after birth. Therefore, if there is hair present after birth, the window of detection is relatively long.

In children (and adults), the hair growth cycle continues, with each phase having a distinct length of time. The growth of each hair follicle is independent, with approximately 85% of all head hair follicles in the anagen phase (growing phase) at any given time. The remainder of hair follicles are not in the growing phase, and this must be taken into account when interpreting any nonneonatal hair results, because drug incorporation does not occur during the resting phase.

Routes of incorporation of compounds into hair include direct incorporation of a chemical via the capillary networks of the hair follicle, as a result of secretions from the sebaceous and sweat glands, and as a result of environmental or external exposure. Measuring and interpreting biomarkers in hair must properly take these different routes of incorporation into account.

The many factors that determine the concentration of a drug in hair should also be considered. These factors include hair color (melanin content), physicochemical properties of the drug, and cosmetic treatment of hair. For example, it has been found that drugs preferentially incorporate into darker hair, most likely because of the relative amounts of eumelanin. Also, physicochemical properties including lipophilicity, basicity, and membrane permeability affect the ability of drugs to incorporate into hair. Generally, basic, lipophilic drugs tend to accumulate more readily into hair samples than more acidic or polar drugs. Cosmetic treatment of hair has been found to decrease hair concentrations of drugs, mainly because of increased hair damage and the removal of hair color pigment. Hair damage causes drug molecules to be lost more easily from the matrix, whereas removal of pigment reduces the amount of melanin to which the drugs are bound. As a result, clinical interpretations of drug concentrations in hair must take these factors into account.

Hair samples are typically collected from the posterior vertex, because hair from this area of the scalp shows the most constant rate of growth. Analysis of hair provides long-term information on an individual’s drug use or exposure. Taking advantage of the uniform growth rate of human hair, approximately 1 cm/mo, it is possible to segment hair samples to more accurately assess the time and pattern of use or exposure. The window of detection for nonneonatal hair samples is therefore dependent on hair length.

**Meconium**

The first few bowel movements of a neonate are composed of meconium. This highly complex matrix begins to form approximately during the 12th week of gestation and
consists of water, gastrointestinal tract epithelial cells, bile acids and salts, enzymes, sugars, lipids, intestinal secretions, and swallowed amniotic fluid. Fetal swallowing of amniotic fluid is the mechanism believed to concentrate compounds within meconium as fetal urine is deposited into the amniotic fluid and is subject to swallowing again. Determining factors of drug incorporation into meconium and the extent of their concentration are mainly determined on the ability of the drug to cross the placenta. Most drugs are able to transfer across the placenta, and the rate of transfer is then determined by molecule size, ionization state, lipophilicity, and protein binding. Because most drugs are small enough to transfer via passive diffusion, the major limiting factor, in terms of drug transport to the fetus, is placental blood flow. Once meconium is formed in the fetal intestine, it is considered a physically static matrix, becoming a record of fetal exposure to the drugs in question during the second and third trimesters of pregnancy. A positive meconium test indicates intrauterine exposure during the second and third trimesters, but is unable to show time or pattern of use.

Dose-response relationships are difficult to determine using meconium samples, mainly because of urine contamination. If fetal exposure occurs close to term and the compound is incorporated into the urine, contamination of meconium can occur once urine is evacuated into a soiled diaper. This situation increases the sensitivity of meconium testing because of the increased compound levels in the sample. However, it could affect the ratio of drugs and metabolites in the sample, and the development of dose-response relationships.

Collection of meconium specimens is easy and noninvasive. Because it is discarded material and there is usually sufficient quantity for analysis, this matrix is practical and useful. Ninety-nine percent of infants pass their first meconium within 48 hours, giving this matrix a wider window of detection than blood or urine. Once 48 hours have passed, it is necessary to evaluate the texture and odor of the sample to determine whether it still meconium or has changed to postnatal feces. The time allowed for sample collection, 2 days, may be seen as limited, but if the neonate is at high risk for drug or alcohol in utero exposure and in hospital care, obtaining a viable sample is not problematic. Collected samples should be minimally 0.5 g, to provide sufficient sample for all analyses. Storage of samples for analysis should be at −20°C or −80°C.

BIOMARKERS FOR ALCOHOL USE

Alcohol exposure during pregnancy can have many serious consequences for the offspring, including birth defects and deficits in cognitive performance and mental development. The fetal alcohol spectrum disorder (FASD) is an umbrella term, which describes the consequences associated with prenatal alcohol exposure. Affecting approximately 1% of all live births in North America, FASD is a major social and economic burden, which can be minimized by early diagnosis and disease management. The clinical presentation of the disease is inconsistent, some lacking evidence of central nervous system neurodevelopment abnormalities. Typically, for the definite diagnosis of FASD, confirmation of prenatal ethanol exposure is needed because some of the characteristics of the disease, in particular physical, may be absent.

Detection of ethanol or its aldehyde has a limited window because they are both rapidly cleared from the blood. A lack of relationship between maternal ethanol consumption and maternal ethanol levels also limits their use to provide an objective analysis. Ethanol itself is not readily incorporated into matrices because of its volatile nature, limiting the window of detection to the hours leading up to delivery, when many
alcohol-dependent mothers may not drink. Two biomarkers, fatty acid ethyl esters (FAEEs) and ethyl glucuronide (EtG), have been established and are currently used to detect exposure or use of alcohol.

**FAEEs**

FAEEs are nonoxidative metabolites of ethanol, formed through the esterification of ethanol with endogenous fatty acids or fatty acyl-coenzyme A (CoA). These reactions are catalyzed by FAEE synthase or microsomal acyl-CoA:ethanol O-acetyltransferase. In particular, FAEE synthase is present in almost all human tissues, with activity being detected in the heart, liver, lungs, adipose tissue, gall bladder, and pancreas. Depending on the carbon chain length and the location of the double bond, different species of FAEEs can be formed. Most FAEEs are transported by albumin within the blood, and once free, are readily broken down by cellular structures in the blood, liver, and pancreas. Unlike ethanol, FAEEs persist in the body for more than a day after significant alcohol consumption, and are able to accumulate in various matrices. Also in contrast to ethanol, FAEEs are readily metabolized by the placenta and thus do not cross. This characteristic indicates that FAEE levels present in meconium represent fetal ethanol metabolism and thus fetal exposure to ethanol.

Several individual FAEEs are analyzed in samples to detect alcohol exposure or use. Interindividual variation in the amount of specific FAEEs formed can result from genetic variations in the enzymes responsible for FAEE formation, the amounts of specific fatty acids in different diets, the degree of alcohol exposure or consumption, and FAEE synthase enzyme kinetics. Ethyl palmitate, oleate, stearate, and linoleate are the predominate FAEEs found in meconium of ethanol-exposed neonates. The cumulative level of select FAEEs is measured because this provides a redundancy system, resulting in higher efficiency, sensitivity, and specificity. In several studies looking at baseline FAEE levels, infants born to women who did not drink alcohol during pregnancy had low FAEE levels. Because the body produces some ethanol during normal metabolism, FAEEs are detected in individuals who do not consume alcohol, thus requiring a clear cutoff value specific to the matrix for differentiation between heavy-drinking and non-drinking individuals. With respect to meconium, the positive cutoff of cumulative FAEE levels was established at 2 nmol/g meconium. This cutoff has 100% sensitivity and 98.4% specificity for detection of heavy fetal alcohol exposure. However, this cutoff value does not allow differentiating between neonates born to non-drinkers and social drinkers. An important limitation of FAEE meconium testing is that samples excreted later in the postpartum period have higher levels of FAEEs than samples collected earlier for the same infant because of de novo production of alcohol from carbohydrates in the meconium. This situation could lead to false-positive FAEE results, and it is recommended to collect meconium samples within 24 hours to ensure that FAEE results properly reflect in utero ethanol exposure.

The FAEE cutoff value for adult hair was established to be 50 ng/g hair, but at this level would identify not only heavy drinkers but some social drinkers too. Hair FAEE results between 20 and 50 ng/g hair indicate moderate levels (ie, social) of drinking. This FAEE cutoff provides optimal sensitivity and specificity, both 90%. Hair care products can result in increased FAEE levels by causing localized FAEE production on the scalp. Differentiating between external and incorporated FAEEs may not be necessary in neonatal hair samples, because the use of hair care products in neonates is not relevant. To confirm FAEE results in adult hair, a secondary confirmatory test is needed, as discussed later.
EtG

EtG is a minor metabolite of ethanol, formed when ethanol is glucuronidated with activated glucuronic acid instead of water.\textsuperscript{10} Although EtG testing lacks sensitivity in detecting moderate or social drinkers, it is highly specific for heavy alcohol use.\textsuperscript{30} EtG measurements are used to confirm hair FAEE results. As mentioned earlier, increased hair FAEE levels can reflect alcohol abuse, social drinking, or use of ethanol-containing hair care products.\textsuperscript{29} EtG can help rule out ambiguous FAEE results caused by external contamination with alcohol hair products. If a hair sample is also positive for EtG (cutoff of 30 pg/mg hair), this indicates excessive alcohol use during the tested time frame, and the potential influence of hair care products is eliminated.\textsuperscript{29}

BIOMARKERS FOR DRUGS OF ABUSE

Illicit substance use in women of childbearing age has increased over the past 3 decades, with a parallel increase during pregnancy.\textsuperscript{5} Drug use during pregnancy is a risk factor for both maternal and fetal complications.\textsuperscript{31} As well, children exposed to drug use in their environment are susceptible to many adverse outcomes associated with drug use affecting their physical and mental health, as well as their social well-being.\textsuperscript{32} The overall increased use of drugs of abuse has necessitated the development of biomarkers of drug use, obviating the shortcomings of maternal reports, providing clinicians with tools that can detect exposure to or use of these illicit compounds. Biomarkers for individual drugs or xenobiotics, each with unique impacts on the health of the child, are discussed.

Cocaine

Prenatal cocaine exposure is associated with low birth weight, prematurity, spontaneous abortions, stillbirths, and microcephaly. In addition, placenta complications have been described, including placental abruption and increased risk of diminished blood flow to the fetus.\textsuperscript{33} Exposure to cocaine during childhood can increase the risk for hypertension, ventricular arrhythmia, seizures, and intracranial bleeding.\textsuperscript{34} Behavioral problems also present themselves during development to both prenatally and postnatally exposed children. Infants exposed in utero are found to have attention deficits with an increased incidence of attention-deficit/hyperactivity disorder.\textsuperscript{32}

Because of the short elimination half-life of cocaine (50 minutes), detection in blood or urine matrices is limited to a few days after use.\textsuperscript{34} Because cocaine and its metabolites readily accumulate in both hair and meconium, these matrices can provide information on in utero exposure for infants as well as environmental exposure in older children.

Cocaine crosses the placenta via passive diffusion and is almost always found with at least 1 of its metabolites in meconium samples.\textsuperscript{15} The site of metabolite production, either fetal or maternal, is undetermined. The most common cocaine metabolite tested and found in biologic matrices is benzoylecgonine. This metabolite is formed through hydrolysis.\textsuperscript{15} Other metabolites, including norcocaine and cocaethylene, are also used in sample analysis for cocaine.

Using all 4 of these biomarkers allows clinicians to obtain an accurate picture of cocaine exposure and make a correct clinical interpretation. The latter is important because environmental cocaine exposure occurs in different ways, including inhalation of crack cocaine smoke, exposure to cocaine residues on surfaces, and through direct contact with a cocaine-using caregiver. The presence of cocaine in a child without the metabolites that are produced systemically indicates environmental
exposure, with low risk of systemic exposure. If benzoylecgonine is also detected at concentrations at least 10% of cocaine hair levels, it is a strong indication of systemic exposure by the child. The presence of norcocaine indicates higher levels of exposure, because this metabolite is not so readily detected, as is benzoylecgonine.

Cocaethylene is an emerging biomarker that can provide additional information regarding exposure. This metabolite is formed when cocaine and ethanol are present concurrently. By using cocaethylene as a biomarker, alcohol use can be detected without assessing alcohol-specific biomarkers. This strategy has great advantages for detecting a risk of fetal alcohol syndrome in neonates who are positive for this metabolite.

**Opioids**

Opioid use has been reported in 1% to 21% of pregnant women. With increasing rates of opioid use and dependency, the incidence of neonatal abstinence syndrome (NAS) has doubled in the past 5 years in Ontario, Canada. Because most infants born to opioid-dependent mothers suffer from NAS, this has become a real public health issue. NAS is characterized by the presence of central nervous system hyperirritability, gastrointestinal dysfunction, and metabolic, vasomotor, and respiratory disturbances. The treatment required for these symptoms increases the hospital stay to an average of 30 to 40 days for infants, primarily in a neonatal intensive care unit. The recent introduction of buprenorphine has resulted in a decreased hospital stay and shorter duration of treatment of infants. Testing women suspected of using opioids could allow for early treatment of the baby and mother. Although the methadone-treated mother is commonly known to the medical system and social services, expecting mothers addicted to other opioids may go undetected, and use of meconium or hair biomarkers may be the first clue to the poor neonatal adaptation shown by the neonate.

Heroin use during pregnancy may be involved in a cycle of overdose and withdrawal and is associated with complications, including spontaneous abortion, antepartum hemorrhage, and stillbirth. Heroin is rapidly deacetylated to the active metabolite 6-monoacetylmorphine (6-MAM), readily crosses the placenta, and is incorporated into fetal tissues within 1 hour of administration. Because 6-MAM persists in the system for a longer period than heroin, it is used as a biomarker to detect heroin use. Along with heroin metabolism to 6-MAM, detectable levels of morphine can also be produced. In addition, illicit heroin usually contains acetylmorphine, and users commonly top up their heroin with codeine before injection. This practice may complicate the interpretation of test results, because the source of codeine and morphine may be unknown in 6-MAM–positive samples.

Codeine and morphine are commonly tested together because morphine is a metabolite of codeine through CYP2D6 metabolism. Morphine is widely distributed in fetal tissues and its level in meconium has been found to correlate with maternal dose, time, and duration of gestational exposure. Similar to codeine and morphine testing, oxycodone and its metabolite oxymorphone are commonly tested together. Hydrocodone is tested with one of its metabolites, hydromorphone, which is also available as a separate analgesic. It is best to determine which drug was prescribed before interpreting the results, because each of the metabolites is also a prescription drug. Because opioids are not contraindicated during pregnancy and can be used during labor, it is necessary to try to identify all medications administered to the mother to properly assess opioid levels.

**Amphetamines**

This group of drugs of abuse includes methamphetamine (commonly called crystal meth), amphetamine (commonly called speed, also Adderall or Dexedrine), and
MDMA (3,4-methylenedioxy-N-methylamphetamine) (commonly called ecstasy). Case reports have reported congenital abnormalities, including heart defects and cleft palate, in infants exposed to amphetamines during the first trimester. All of these compounds have been associated with similar impacts on behavior and cognition of exposed children, including lower IQ scores, difficulties with advancement in school, and physical fitness activities. In addition, children of addicted mothers have been found to show more behavioral problems. Each compound in this group has its own method of detection, and results are used in conjunction to provide a more accurate interpretation.

Methamphetamine is able to cross the placenta at a rapid rate and even at lower levels on the fetal side; it persists because of slower elimination, resulting in prolonged fetal exposure. The fetal elimination rate of amphetamine is also reduced, but to a greater extent than that of methamphetamine. The prolonged elimination of both methamphetamine and amphetamine can cause accumulation of both compounds on the fetal side if the mother is using these compounds on a consistent basis. Environmental exposure is also a concern for children, especially if methamphetamine is smoked in the household.

Each one of these amphetamine derivatives can be tested for in meconium and hair samples to assess exposure in children. Interpretation of samples positive for amphetamines can become complex depending on which compounds are detected. If either methamphetamine or amphetamine is detected alone, this indicates exposure or use of this compound. Samples positive for both methamphetamine and amphetamine can be interpreted in 3 ways. First, the amphetamine could be a product of methamphetamine metabolism, indicating methamphetamine use or exposure. Second, seized illicit methamphetamine contains amphetamine as well, indicating methamphetamine use and, unknowingly, amphetamine exposure. Third, it can suggest both methamphetamine and amphetamine were used during the tested time frame. It is necessary to test for all amphetamine compounds if exposure is suspected, because the interpretation of amphetamines can be complex.

Cannabinoids

No clear increase in pregnancy complications for users of marijuana is known. However, some studies have associated marijuana use during pregnancy with lower birth weights and longer gestations. Users of marijuana have been documented to use other illicit drugs, which increases the risk of complications. In addition, it was recently found that children exposed to marijuana had lower performance on standardized tests, indicating that long-term behavioral and neurodevelopmental issues may occur in these children.

Cannabis sativa, marijuana, produces the group of cannabinoids, with more than 50 unique compounds. Δ9-tetrahydrocannabinol (THC) is the primary compound of the cannabinoids and is metabolized to the active compound 11-hydroxy-Δ9-tetrahydrocannabinol and subsequently 11-nor-Δ9-tetrahydrocannabinol-9-carboxylic acid (THC-COOH). Analysis of samples for cannabinoid content is primarily conducted using enzyme-linked immunosorbent assay (ELISA) techniques and yields a qualitative result of positive or negative depending on the cutoff value set for the method. Because of the commonality of infrequent cannabis users and their chance of increasing false-negative results, the sensitivity of the immunoassay analysis can vary depending on the tested population. Confirmation of ELISA results can be conducted using gas chromatography-mass spectrometry, but because of the nature of THC and the extraction methods used, which alter the ratio between THC-COOH and total cannabinoids, lower rates of confirmation have been found. Positive ELISA
results for cannabinoids indicate use or high exposure, because THC and its metab-
olites are not readily incorporated into matrices. Implementing liquid chromatography-
mass spectrometry analysis is a potential method for THC analysis, because it is able
to detect lower levels of compounds.

BIOMARKERS OF ENVIRONMENTAL EXPOSURES

Although there are numerous biomarkers for a multitude of environmental chemical
exposures, we use 1 powerful example of environmental exposure through diet
primarily to show the importance of biomarkers in this field.

Methylmercury

Methylmercury is a known neurotoxin produced from inorganic mercury by anaerobic
organisms that live in aquatic environments. Because methylmercury strongly binds
to cysteine-containing proteins, it is not readily eliminated from the body; its elimina-
tion half-life is approximately 60 days, and it can also accumulate in the environ-
ment. Fish consumption is the main source of methylmercury ingestion, with
a dose-response relationship between the amount of fish consumed and the total
systemic mercury burden, which can be measured in hair and blood. Methylmercury
readily crosses the placenta and because the blood-brain barrier does not fully
develop until 6 months after birth, prenatal exposure can result in adverse neurodeve-
lopmental effects. These adverse neurodevelopment effects can include deficits in
the functional domains of language, memory, and attention. Adverse effects of
high prenatal exposure to methylmercury have been well documented because of 2
serious poisoning events in Japan and Iraq. In addition, neurologic damage can
also occur in children and adults who consume fish containing high mercury levels
on a regular basis. This damage is often characterized by ataxia, sensory distur-
bances, and mental state changes.

Total mercury levels are commonly tested in hair samples, both maternal and fetal.
Data show that methylmercury makes up 80% of the total mercury detected in hair,
with the remaining 20% being inorganic mercury. With respect to maternal hair,
levels as low as 10 µg/g hair have been associated with severe adverse effects to
the fetus. In a recent meta-analysis, the lower observable adverse effect level of
mercury for adverse fetal outcome was defined at 0.3 µg/g maternal hair. Analysis
of hair of women who consume more than 340g (12oz) of fish per week could be
a useful tool to assess whether this consumption is associated with increased mercury
levels. If so, these women may wish to modify their diet before becoming pregnant,
decreasing the body’s burden of mercury and ensuring minimal fetal exposure to
methylmercury.

IMPORTANCE OF BIOMARKERS

Substance abuse, prenatal or not, is an ongoing public health concern, affecting not
only the user themselves but also their families, and the health system economically.
Beyond being biomarkers for physical and neurologic well-being of exposed children,
hair and meconium measures identify women who continue to use drugs of abuse or
alcohol despite being aware of their pregnancy. Children raised by an addicted mother
have increased risk for neglect and abuse. Behavioral problems associated with
exposure to drugs of abuse can commence in utero, but the quality of the postnatal
environment can also modify the child’s behavior and development. Hence, these
biomarkers constitute strong predictors of environmental risk for the child, and
a need for close follow-up of the well-being of the child. Identifying abuse is critical
to allow social workers and children’s aid societies to implement the necessary interventions and provide the best environment possible for the child.

Polydrug use is commonly reported in those who tested positive for certain drugs. In terms of opioid-dependent women, benzodiazepines, cocaine, and marijuana are the most common concomitantly used drugs.36 This finding was confirmed by showing that meconium samples positive for opioids were likely to also be positive for cocaine, benzodiazepines, methadone, and FAEEs in a previous study.37 As well, stimulant use of amphetamines and cocaine by the mother was a potential risk factor of alcohol use and subsequently fetal alcohol exposure.19 Knowing which drugs are often used concomitantly can allow clinicians to potentially diagnose drug exposures in children that otherwise would have gone undetected.

Environmental exposures to chemicals are increasingly becoming more publicly acknowledged. Developing biomarkers for specific environmental toxicants can help clinicians detect both high-level and low-level exposures in humans. Detection can then lead to a decrease in exposure to these toxicants, and possibly discontinuing public use of compounds causing major adverse effects on health.

SUMMARY

The impact that drug and alcohol use or abuse during pregnancy can have on neonates and infants is serious. Because mothers may grossly underreport use because of fears and embarrassment, identification of biomarkers in newborns that point to such use has become a major research focus and is becoming more commonly accepted and used in clinical practice. Using such biomarkers provides clinicians with an opportunity to appropriately diagnose and, where possible, treat newborn conditions associated with prenatal alcohol or drug abuse. It also provides social workers with the opportunity to implement necessary interventions to provide a safer environment for the child. Similarly, the development of effective biomarkers to detect environmental chemical exposures allows the health care team to implement, where available, mitigating interventions to minimize their adverse effects.

REFERENCES


35. Natekar A, Koren G. Interpretation of combined hair fatty acid ethyl esters, cocaine and cocaethylene. Ther Drug Monit 2011;33:284.


